#24 Decl. 4/9/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Johnson et al.

Group: 1648

Serial No. 09/485,512

Examiner: U. Winkler

Filed: May 5, 2000

For: RECOMBINANT PORCINE ADENOVIRUS VECTOR

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents, Washington, D.C. 20231 on _____ Kay Speaker

DECLARATION OF DR. JEFFREY MICHAEL HAMMOND
37 C.F.R. § 1.132

1. Background of declarant

I am a co-inventor of the above-captioned application. I have over 25 years of experience in the field of animal health, including research, development, and applications in the areas of virology, diagnosis, epidemiology, immunology, and molecular biology. I have expertise in the development of adenoviral vectors and vaccines, particularly in the development of porcine adenovirus recombinants. I obtained an Honours Degree in Immunology from the North East Surrey College of Technology in London. Through London University, I completed a PhD in Virology and Molecular Biology focusing on African swine fever virus and construction of vaccinia virus recombinants. I am currently a Senior Research Scientist within the Vaccines and Therapeutics Program at the Commonwealth Scientific & Industrial Research Organisation's Australian Animal Health Laboratory in Geelong, Victoria.

2. Purpose of the declaration.

This Declaration presents, for the Examiner's consideration, various facts in support of nonobviousness of pending claims 1, 2, 4, 25-32 and 39-62 of the above-captioned invention.

3. Differences between human and porcine adenoviruses.

There are significant differences between human adenoviruses and porcine adenoviruses. Due to such differences, there was no reasonable expectation of success in applying the teaching

of the cited references Callebrant et al, Torres et al, Kleibocker and Reddy et al to the construction of a recombinant porcine adenovirus for the expression of a heterologous sequence as claimed herein. Some of the differences between human adenoviruses (HAV) and porcine adenoviruses (PAV) are described below:

Compared with human adenoviruses, PAV3 differs considerably in its genome sequence. In this regard, sequence homology comparisons between PAV3 and human adenovirus serotypes HAV2 and HAV5 are described herein.

At the genome level, PAV3 has only 54.9% and 54.8% identity with HAV2 and HAV5, respectively. At the protein level, the comparisons also show significant differences. The PAV3 fiber protein has only 24.2% and 23.1% identity with fiber of HAV2 and HAV5, respectively. The PAV3 penton protein has only 58.1% and 58.2% identity with penton of HAV2 and HAV5, respectively. The PAV3 hexon protein has only 66.9% and 66.8% identity with hexon of HAV2 and HAV5, respectively.

These analyses have been made based on the disclosures of GenBank accession numbers:

- J01917 Adenovirus type 2, complete genome
  gi|209811|gb|J01917.1|ADRCG[209811]
- M73260 Mastadenovirus h5 gene, complete genome
  gi|209842|gb|M73260.1|ADRCOMPGEN[209842]
- AF083132 Porcine adenovirus 3 strain 6618, complete genome
  gi|4092658|gb|AF083132.1|AF083132[4092658]
- Pearson, W.R., Wood, T., Zhang, Z., and Miller, W. (1997)
  Comparision of DNA sequences with protein sequences, Genomics 46: 24-36.

The facts clearly show that PAV3 is very different to HAV in both genome and protein sequence content. The mere relationship of being in the same virus family or the sharing of structural and functional features does not provide one skilled in the art with the ability to manipulate a comparable sequence at the molecular level and, given the state of the art at the time of the invention, to predict with reasonable success the structure and function of expressed proteins. This evidence of the low level of sharing of genetic and protein sequences profoundly

reduces the expectation of success for applying the structural and functional features of one family member to another.

4. The invention includes embodiments of vectors having sizes above and below the level of 105% of the wild-type genome size.

The claim limitation of "greater than 105% the size of wild-type adenovirus" is not necessary in part due to the nonobviousness of a claim lacking the limitation. The feature of a genome size greater than 105% of the wild type genome is merely an embodiment showing the superior stability of the porcine adenovirus of the present invention compared with known human adenovirus vectors or compared with anything that could have been constructed with a reasonable expectation of success in light of the combination of references cited. This assertion is supported by the facts herein reflecting the lack of a reasonable expectation of success, the long-felt but unsolved needs in the field of the invention, and the failure of others to make the invention.

As evidence of developing recombinant porcine adenovirus vectors without a limitation that a vector need exceed the 105% level, I have developed vectors having less than 105% of the wild-type total genome size. In the attached Exhibit A, I demonstrate the successful generation and use of three examples of such vectors. In Example 1, the recombinant porcine adenovirus vector expresses porcine interferon-gamma. In Example 2, the recombinant porcine adenovirus vector expresses porcine interleukin-5. In Example 3, the recombinant porcine adenovirus vector expresses porcine granulocyte colony stimulating factor. In Examples 1, 2, and 3, the size of the recombinant vector is 103.4%, 103.1% and 103.6% of wild-type, respectively.

5. The difficulties encountered in making the invention further rebut any reasonable expectation of success and provide evidence of unexpected results.

The Office Action of 1 October 2002 twice asserts that the cited references provide a "high expectation of success" in making the claimed invention. First, the Office Action on page 6 states:

> Two of the references utilize human adenovirus for the development of porcine vaccines, because the viruses belong in the same family and therefore share important structural and functional features the ordinary artisan would have had a high expectation success in utilizing the teachings from the human adenoviral vectors vaccine and apply the same construction for the expression of a heterologous sequence in a porcine adenovirus.

Second, the Office Action on page 7 states:

Therefore, given what is known in the art regarding the construction of adenoviral vectors and the knowledge of structural proteins that provide protective immunity against hog cholera virus in an animal the ordinary artisan would have a high expectation of success when inserting the Hog cholera virus structural proteins into an adenoviral vector.

Following is a description of significant difficulties in making the claimed invention which support the assertion that while it may have been obvious to try, the expectation of success was not reasonable and moreover not high.

The construction of the major late promoter-leader sequences expression cassette has a high degree of technical difficulty. The Reddy et al. paper clearly states that PAV does not have a Tri-partite leader sequences thus teaching against the present invention [Reddy, P.S., Nagy, E., Derbyshire, J.B. (1995) Sequence Analysis of putative pVIII, E3 and fibre regions of porcine adenovirus type 3.Virus Research 36, 97-106.] The PAV MLP promoter of about 250bp and the first leader sequence, 83bp are located together. However, the second (68bp) and third leaders (99bp) are located separately and distantly from the promoter. That is, they are spread over a 6 kilobase region. Knowledge of particular HAV sequences in this context is of no value as applied to PAV sequences. As taught in the Specification of the above-captioned application, the construction of restriction maps enabled the approximation of the location of the promoter. Subsequently, fragments were cloned and sequenced.

One region appeared to have promoter elements that were suggestive of the MLP and the first leader. To locate the other two leader sequences, message RNA from late times in infection was isolated and cDNA clones isolated. To clone the three leader sequences it was necessary to first locate late genes (hexon and penton), sequence them and their 5' non-coding sequences from which primers were designed in order to allow separate amplification of the second and third leaders. The promoter was located and sequenced and the inventors designed a primer in the MLP cap site from this information to complete the PCR amplification of the three leaders. There was no sequence data available at the time, and the combination of cited references did not teach how to do this. In combination, the teachings of the references cited in the Office Action did not create a reasonable expectation that the inventors' efforts would be successful. All these elements were then assembled, which took a high degree of technical expertise. Care was taken to ensure that the promoter and the leaders were joined such that splice donor and acceptor sites were not disrupted, as failure to do so would have resulted in a non-functional promoter. The combination of references cited did not provide information or the reasonable expectation of

success for the necessary elucidation of the sequence and location of the MLP and tri-partite leader elements included in the present invention.

In addition, it was necessary to determine how much of the upstream promoter was required for correct function. None of the locations of the leader elements could be predicted or taught from knowledge of HAV; the sizes of the leader sequences were different, and their splice consensus sequences were different. None of this could be predicted and, therefore, anyone of ordinary skill in the art would have to undertake considerable experimentation to overcome these difficulties without having a reasonable prospect of success. After the promoter leader sequences had been assembled, they were tested for activity. The CAT reporter gene was made and used with expression assays performed on cells transfected with the plasmid construct to confirm promoter-leader activity.

As to the generation of recombinant viruses, a difficulty the inventors had to overcome was the choice of cell line in which to generate recombinant viruses. It was found that a continuous cell line (PK15) was ineffective in the generation of recombinants. This cell line supports PAV replication, but did not allow the generation of recombinant virus. A recombinant is formed when a cross-over event occurs between the right-hand cloned plasmid containing the expression cassette and the genomic left hand end DNA. This is dependent on the efficiency of transfection of these DNA sequences into cells. The inventors hypothesized that the PK15 cell line was not able to be efficiently transfected with the required PAV DNA sequences. It was then necessary to test a range of pig cell lines and primary pig kidney cells were chosen. It was found that the use of this cell line overcame the problem of efficient transfection and resulted eventually in the generation of a recombinant. The choice of cell line was not obvious; as PAV grows well in PK15 cell line, it would follow that recombinants should be able to be generated. This was not the case. This surprising discovery has resulted in the inventors changing their usual method of making recombinants, now using primary cells as disclosed.

6. The invention satisfied a long-felt but unsolved need in the art.

a. First, the need for a stable porcine adenovirus vector was a persistent one that was recognized by those of ordinary skill in the art. I am personally aware of such a long-felt need. This long-felt need is further supported by the activity in the field as reflected by the references cited. Second, the long-felt need had not been satisfied by others before the present invention. This lack of satisfaction persisted despite the activity in the field; others of ordinary skill in the art were working on the problem. Furthermore, there is evidence that persons skilled in the art who were working on the problem knew of the teachings of the references (where such

knowledge was present due to the fact that some of the skilled artisans had generated the cited references and in some cases referred to both HAV and PAV information) and were still unable to solve the problem. Stable recombinant porcine adenovirus vectors along with related methods of producing such vectors and methods of vaccinating using such vectors were not available until made so by virtue of Applicant's disclosure. Third, the invention satisfies the long-felt need by making available stable recombinant porcine adenovirus vectors along with related methods of producing such vectors and methods of vaccinating using such vectors.

Despite considerable activity in the field, technical difficulties such as those described herein meant that it took Applicants from 1992 to 1997 to generate the first recombinant. None of the information in Reddy et al. paper would allow this, nor is anything ir the Reddy et al. paper or other references in combination sufficient to allow the generation of recombinant PAV viruses. It is noteworthy that Reddy failed to generate a recombinant until 1999. [Reddy, P.S., Idamakanti, N., Hyun, B.-H., Tikoo, S.K. and Babiuk, L.A. (1999). Development of porcine adenovirus-3 as an expression vector. J Gen Virol 80, 563-570] Thus it took him at least four years, he did not succeed until after Applicants' disclosure. Such period is a considerable time and Reddy required considerable experimentation. This is further an independent indication that any combination of references involving information from HAV did not teach how to make a PAV recombinant and that such a combination did not render the present invention obvious.

7. Declaration is of own knowledge and true.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jeffrey Michael Hammond

3/3/03
Date

Exhibit A
of
Declaration of Dr. Jeffrey Michael Hammond

Examples of Recombinant PAVs of less than 105% total genome size containing cytokine genes.

Example 1: Recombinant porcine adenovirus expressing Porcine interferon-γ

A recombinant porcine adenovirus expressing the porcine interferon-γ gene was constructed (rPAdV-IFN-γ) and shown to be positive for expression of IFNγ by bio-assay (fig 1). This rPAdV was then tested in safety trials designed to detect recombinant virus shedding from vaccinated outbred pigs. No recombinant virus was re-isolated following 3 serial passages of nasal or rectal swabs in tissue culture and no rPAdV DNA was detected by PAdV specific PCR on the passaged material (data not shown).

Ten outbred 5 week old weaned pigs were inoculated sub-cutaneously with a single dose of $2x10^4$ $TCID_{50}$ rPAdV-IFNγ. Groups of control pigs were either left un-treated or given an equivalent single dose of rPAdV-gp55 as recombinant PAdV controls. Pigs were fed a standard diet of commercially available medicated feed and weights recorded weekly. Mean weights for each group are shown in table 1.

A second trial was carried out using the same vaccination protocol and experimental conditions as above, however, pigs were fed on a diet of non-medicated feed containing no added antibiotics. Weights were again recorded weekly and the mean weights for each group are shown in Table 1.

In both medicated and non-medicated feed trials it was demonstrated that treatment of pigs with a single dose of rPAdV-IFN-γ resulted in higher proportion of pigs reaching a defined target weight by day 68 which is the end of the post-wean period. Both control groups, either untreated or treated with the recombinant PAdV expressing the gp55 antigen, presented with similar lower proportions of pigs reaching the weight target. Of great interest was the observation that in the non-medicated feed trial there was a 20-30% increase in the number of pigs reaching the target weights than in the control groups.

Statistical analysis of the mean weights demonstrated that this increase was significant ($p<0.05$) compared with both control groups.

The data indicate that treatment of pigs with one dose of rPAdV-IFNγ at weaning, results in significant weight gains to the end of the post-weaning period in the order of 7-8%, both in pigs fed on medicated or non-medicated feed.

A further observation from this experiment was that pigs treated with one dose of rPAdV-IFNγ were more similar in their growth rates throughout and as such had reduced weight variation between individual animals compared with both groups of controls. The implication of this observation is that pigs treated with rPAdV-IFNγ will be more uniform in their growth rate and reach target weights sooner than untreated groups.

Table 1: % of pigs reaching target weights

	non-medicated feed			medicated feed		
	>20kg day 54	>20kg day 61	>25kg day 68	>20kg Day 54	>20kg Day 61	>25kg Day 68
controls	10	60	60	0	60	40
1 dose	0	90	80	0	60	60
2 dose	10	70	70	10	30	30
gp55	10	50	50	0	60	40

Table 2: Comparison of the Day 68 mean weights

PAV-IFN trials			PAV-IL5 trials		
	Med Day 68	Non-Med Day 68		Med Day 68	Non-Med Day 68
control	23.74	24.95	controls	24.2	23.57
			IL5 inj	24.58	24
IFN 1 dose	**26.02**	**26.35**	1 dose	24.3	**24.68**
IFN 2 dose	24.02	25.47	2 dose	**25.68**	24.52
gp55	24.8	24.04	gp55	24.15	22.18

Example 2: Recombinant porcine adenovirus expressing porcine interleukin 5

A recombinant porcine adenovirus expressing the porcine interleukin 5 gene was constructed (rPAdV-IL5) and shown to be positive for expression of IL5 by biological assay (Fig 2). This rPAdV was then tested in safety trials designed to detect recombinant virus shedding from vaccinated outbred pigs. No recombinant virus was re-isolated following 3 serial passages of nasal or rectal swabs in tissue culture and no rPAdV DNA was detected by PAdV specific PCR on the passaged material (data not shown).

Ten outbred 5 week old weaned pigs were inoculated sub-cutaneously with a single dose of $2x10^4$ $TCID_{50}$ rPAdV-IL5 or another group was given two doses 2 weeks apart, and a third group was injected with purified IL5 protein, 100μg per dose twice weekly for 5 weeks. A group of control pigs were un-inoculated and another control group was vaccinated with an equivalent dose of a recombinant PAV expressing the gp55 gene of Classical swine fever virus (rPAV-gp55).

Pigs were maintained on weaner ration either containing antibiotics (medicated) or no antibiotics (non-medicated). Weights were monitored weekly and expressed as the proportion of pigs in the group reaching a target weight at a defined time.

Table 3 shows the results and indicated that pigs treated with rPAV-IL5 showed a higher proportion of pigs reaching target weights compared to controls or to injected IL 5 protein.

Table 3: % pigs reaching target weights treated with PAV-IL5

	Non-medicated feed			medicated feed		
	day 54	day 61	day 68	day 54	day 61	day68
Group	>15kg	>20kg	>25kg	>15kg	>20kg	>25kg
controls	60	60	20	30	20	30
inj IL5	60	60	30	40	60	50
1 dose	80	70	40	30	30	30
2 dose	80	70	40	70	60	50
gp55	30	30	20	40	30	30

Statistical analysis of the mean weights (Table 2) demonstrated that there was a significant ($p<0.05$) increase in weights in the medicated 2 dose rPAdV-IL5 group compared with both controls. The increase in weight gain was approximately 5-6%.

Statistical analysis of the mean weights demonstrated that there was a significant ($p<0.05$) increase in weights in the non-medicated 1 dose rPAdV-IL5 group compared with both controls. The increase in weight gain was approximately 10%.

Example 3: Porcine Granulocyte Colony Stimulating Factor:

A recombinant porcine adenovirus expressing the porcine G-CSF gene was constructed (rPAdV-G-CSF). This construct was tested in safety trials designed to detect recombinant virus shedding from vaccinated outbred pigs. No recombinant virus was re-isolated following 3 serial passages of nasal or rectal swabs in tissue culture and no rPAdV DNA was detected by PAdV specific PCR on the passaged material (data not shown).

Two groups of 8 outbred, 5 week old, pigs were housed in adjoining pens. All pigs were bled daily from day 0 (14 days before vaccination) until the end of the experiment (day 37) and their white blood cell counts recorded. One group was vaccinated subcutaneously with $1x10^5$ $TCID_{50}$ rPAdV-G-CSF on day 14. The second group of 8 pigs were left un-treated. At day 14 post vaccination all pigs were challenged intra-tracheally with 106 colony forming units/ml of *Actinobacillus pleuroneumoniae* (HS93 serovar 7) (APP) and monitored daily for clinical signs of APP. Post-mortems were carried out on all pigs on day 12 post-challenge with APP lung lesion scores recorded and general condition noted.

White blood cell analysis

Up to the day of APP challenge there was no significant difference in overall white blood cell counts between the un-treated control pigs and those vaccinated with rPAdV-G-CSF (Figure 3). Surprisingly no increase in peripheral blood neutrophil numbers was observed in the treated group. However, following challenge with APP, significant differences were observed between the 2 groups. Control challenged pigs demonstrated a sharp increase in peripheral blood neutrophils which peaked at 24hrs post infection, dropped considerably and then showed a second peak at 8 days post challenge and remained high until termination of the experiment. This significant increase in circulating neutrophils is the typical response observed following experimental infection of pigs with APP. In contrast, rPAdV-G-CSF treated pigs showed a delayed and more sustained increase in neutrophil levels that did not reach its peak until 96 hrs post challenge. This peak was significantly lower than that of the control pigs and once levels had decreased they did not rise again unlike the un-treated group (Figure 4). It is probable that

the increase in peripheral blood neutrophil levels was a direct result of the APP infection and that these neutrophils were being recruited to the lungs in order to combat the infection, although no cell counts were carried out on lung material.

From day 0 when daily bleeding of pigs began there was a general fluctuation in wbc counts in both groups. However, treatment with rPAdV-G-CSF appeared to reduce this fluctuation with far less variation in wbc counts observed when compared with the un-treated control group. This effect was also apparent following challenge with APP, where increases in wbc populations were less substantial and more tightly constrained than controls (Figure 3).

At termination of the experiment, 12 days post challenge, the condition of all pigs was recorded and scored and at post mortem all pigs were examined for the presence of APP lesions in the lungs. The condition of all control pigs was very poor whereas all rPAdV-G-CSF treated pigs were in good condition showing no clinical signs of disease. At post-mortem, all control pigs showed lesions in the lungs with adhesions. In contrast, all pigs treated with rPAdV-G-CSF had significantly reduced lesions (Table 4). A comparison of the lesion/lobe scores for the 2 groups demonstrated that there was a substantial 94.5% reduction in the score for the rPAdV-G-CSF group. This result clearly demonstrated that a single dose of rPAdV-G-CSF was able to give substantial protection against a severe experimental challenge with APP, confirming that a recombinant PAdV expressing G-CSF is a potent inhibitor of bacterial infection (APP) in pigs.

Table 4: Clinical disease scores for pigs treated with rPAdV-GCSF.

Group 1 controls challenged	% disease (700%)	Lobes affected/7	Group 2 PAdV-GCSF challenged	% disease (700%)	Lobes affected/7
#180	70	2	#186	15	1
#181	70	3	#187	2	1
#182	15	1	#188	5	1
#183	40	2	#189	5	1
#184	140	3	#190	17	2
#185	20	2	#191	0	0
Total	355	13	Total	44	6
Lesion score 1: 355/4200 = 8.5%			Lesion score 1: 44/4200 = 1.0%		
Lesion score 2 : 355/6 = 59.2			Lesion score 2: 44/6 = 7.3		
Lobe score: 13/42 X 59.2 = 18.3			Lobe score : 6/42 X 7.3 = 1.0		

Lesion score 1 is given as a percent per group: total %/(700XN)
Lesion score 2 is given per pig, per group (total % disease/N)
Lobe score is given as [total lobes affected/N(42) X lesion score 2

Figure 1: interferon gamma bio-assay

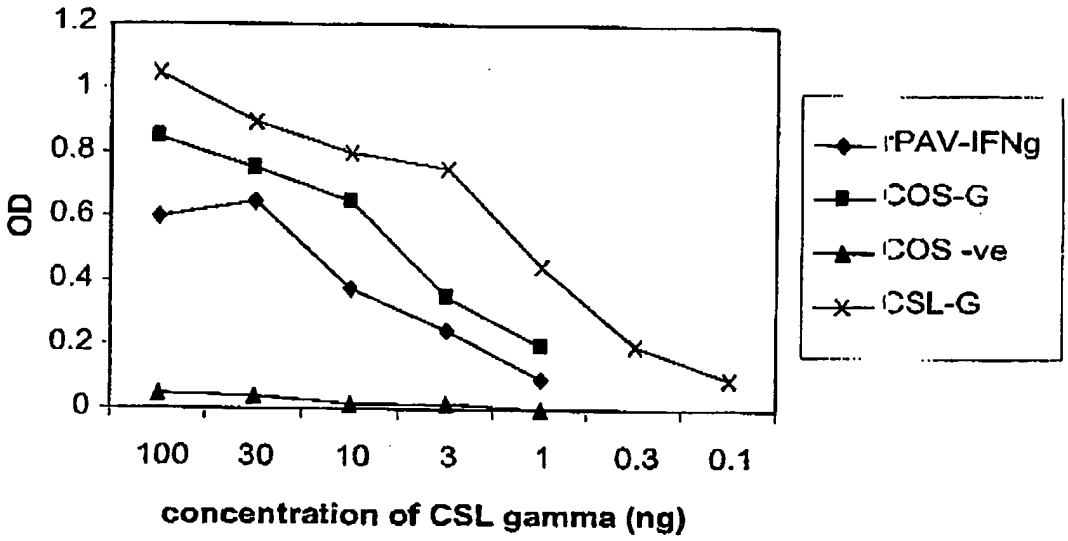


Figure 1: Porcine IFN-γ assay. Supernatants of recombinant PAdV-IFN-γ infected tissue culture were tested in a biological assay for activity compared with COS cells expressing porcine IFN-γ.

Figure 2: IL5 bio-assay

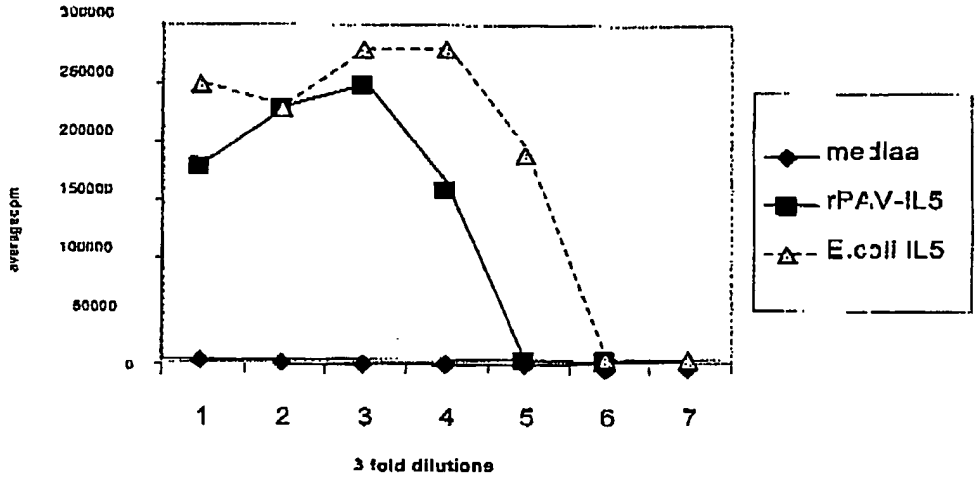
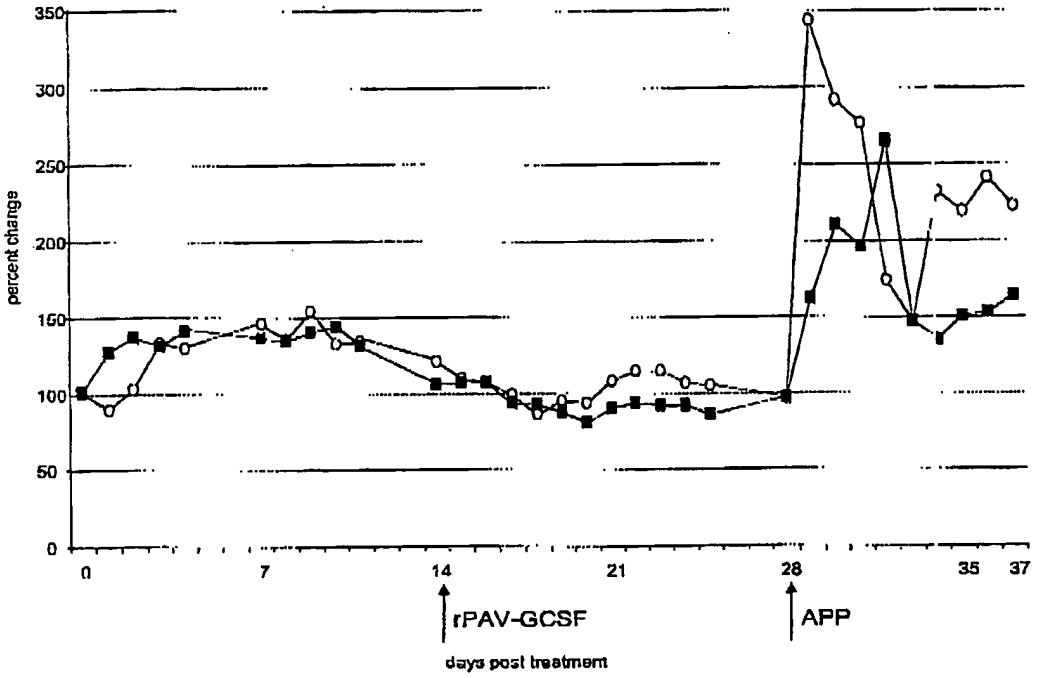


Figure 2: Porcine IL5 assay. Supernatants of recombinant PAdV-IL5 infected tissue culture were tested in a biological assay for activity compared with E. coli expressed porcine IL5.

Figure 4: % Neutrophil change following a single treatment with rPAdV-GCSF and challenge with APP.



■ = rPAdV-GCSF treated

○ = Un-treated

Figure 3: Changes in % white blood cell counts following treatment with rPAV-GCSF single dose, indicated by the arrow (upper panel), or control pigs. The dotted vertica. line indicates the challenge time point with APP.

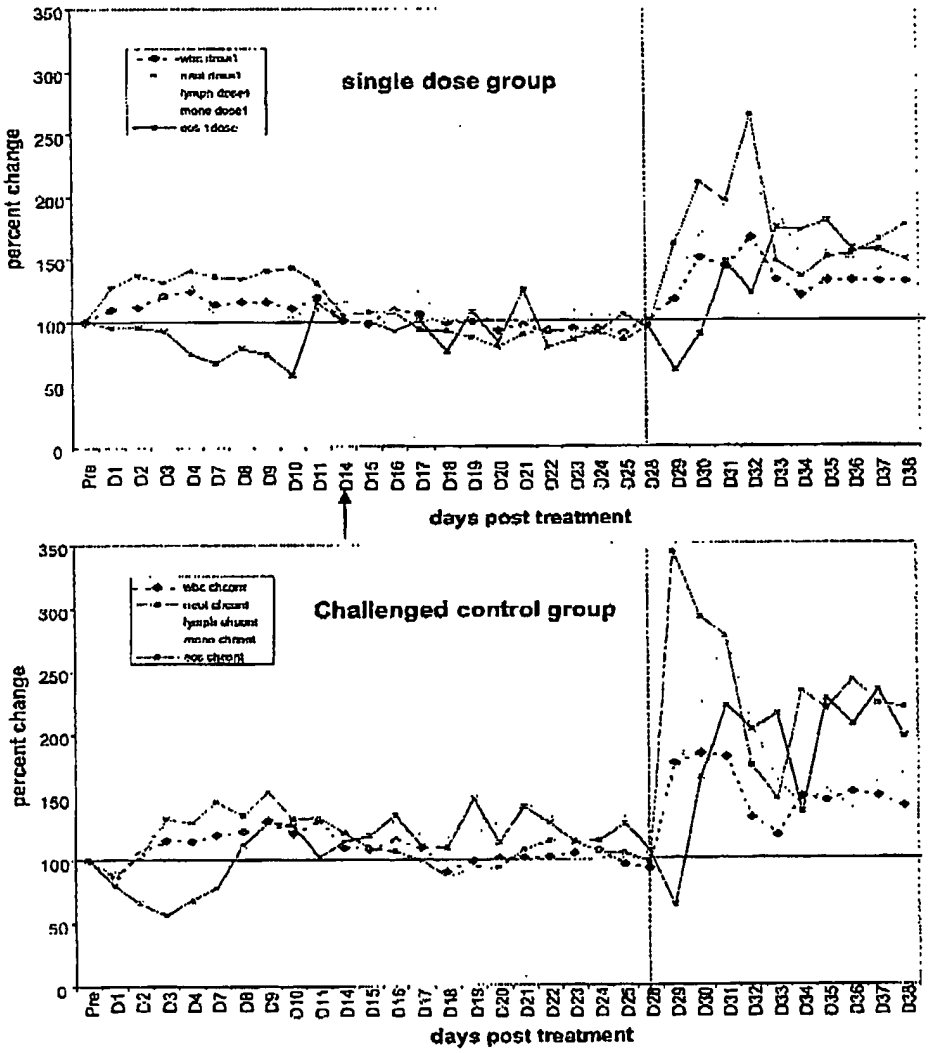


Figure 1: interferon gamma bio-assay

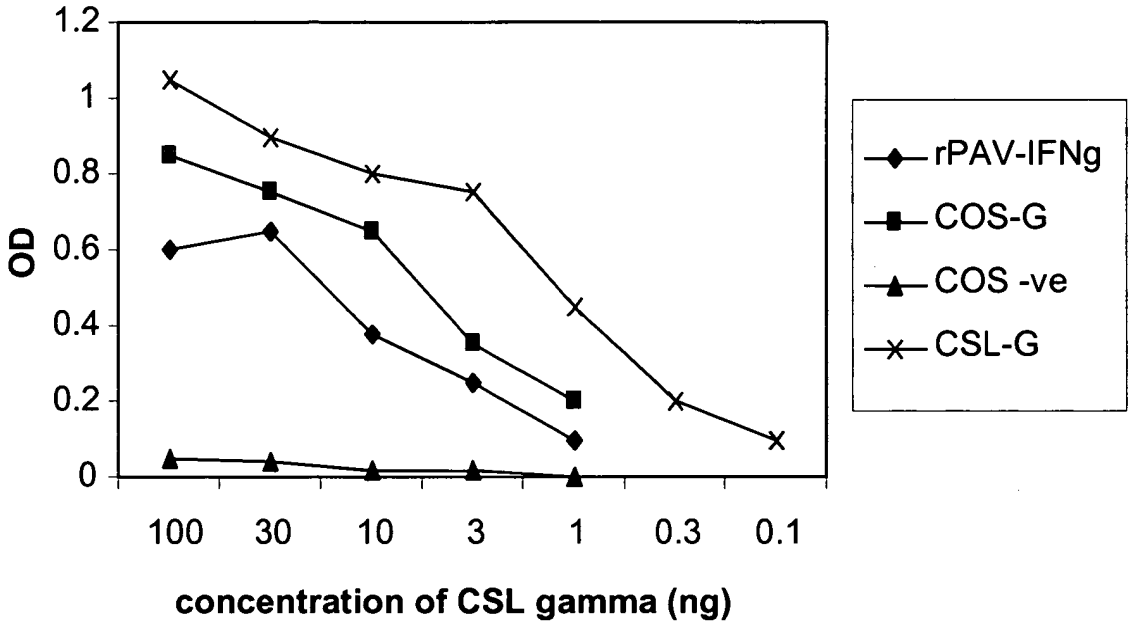


Figure 1. Porcine IFN-gamma assay. Supernatants of recombinant PAdV-IFN-gamma infected tissue culture were tested in a biological assay for activity compared with COS cells expressing porcine IFN-gamma.

Figure 2: IL5 bio-assay

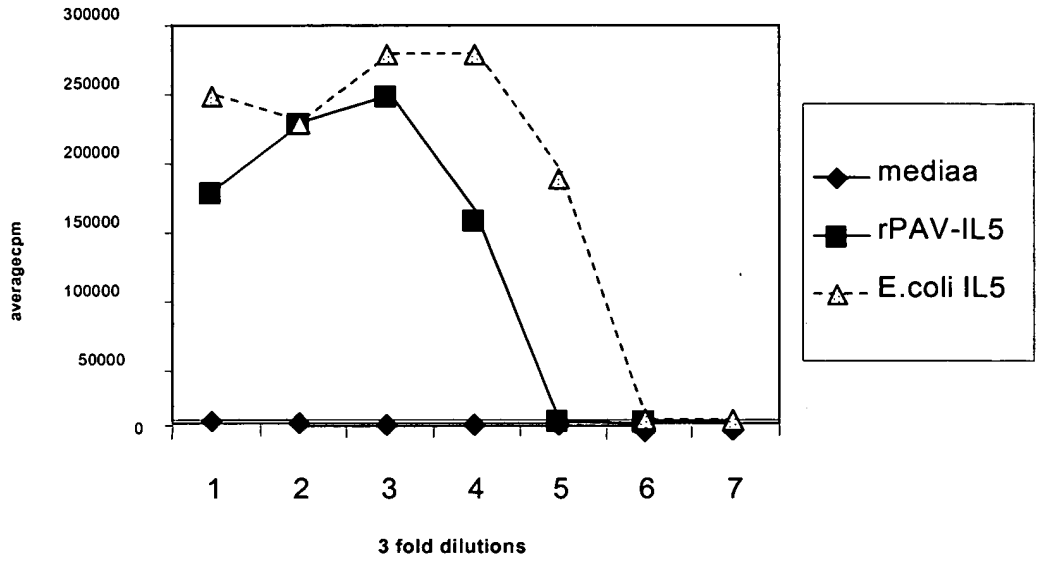


Figure 2. Porcine IL5 assay. Supernatants of recombinant PAdV-IL5 infected tissue culture were tested in a biological assay for activity compared with E. coli expressed porcine IL5.

Figure 3: Changes in % white blood cell counts following treatment with rPAV-GCSF single dose, indicated by the arrow (upper panel), or control pigs. The dotted vertical line indicates the challenge time point with APP.

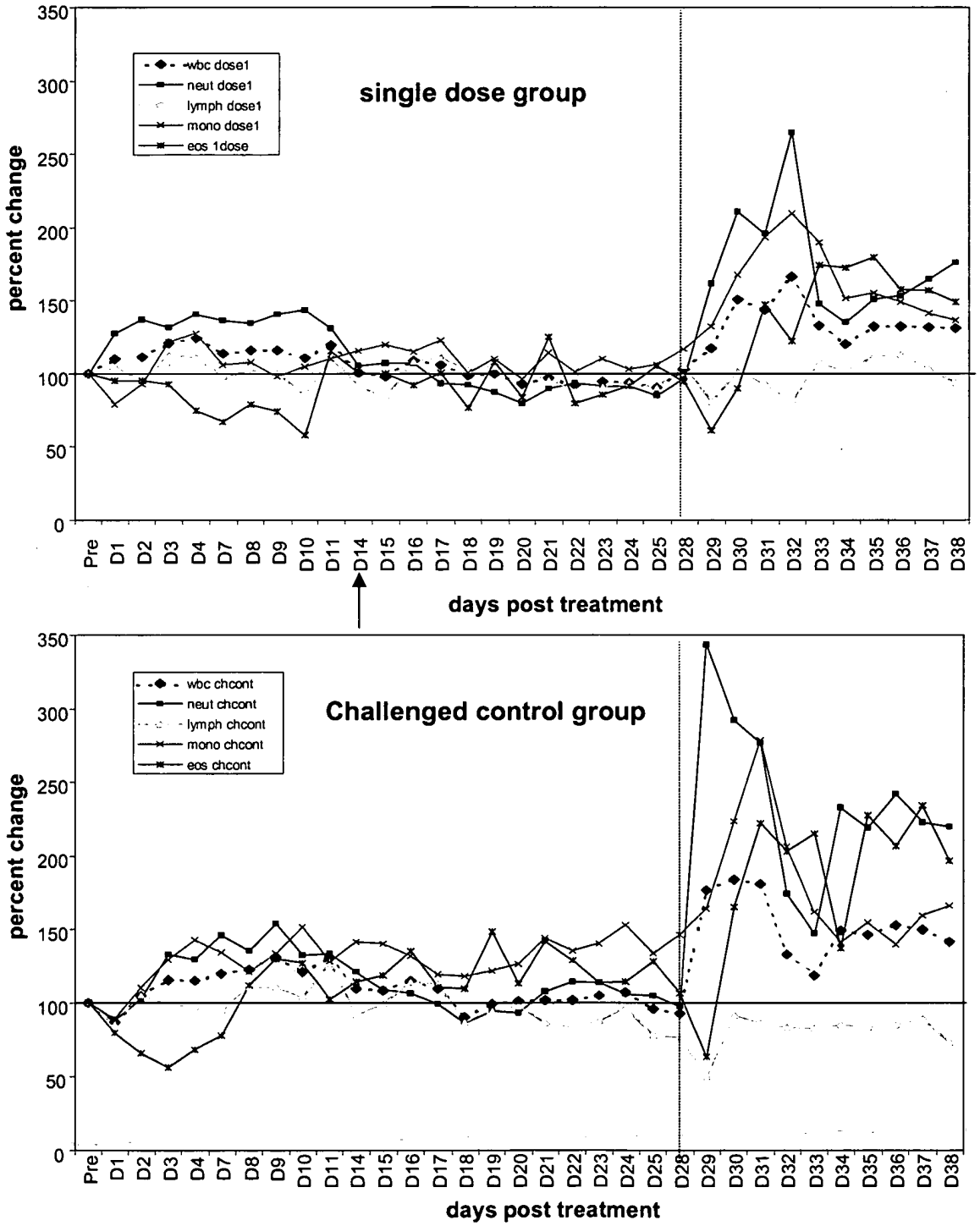
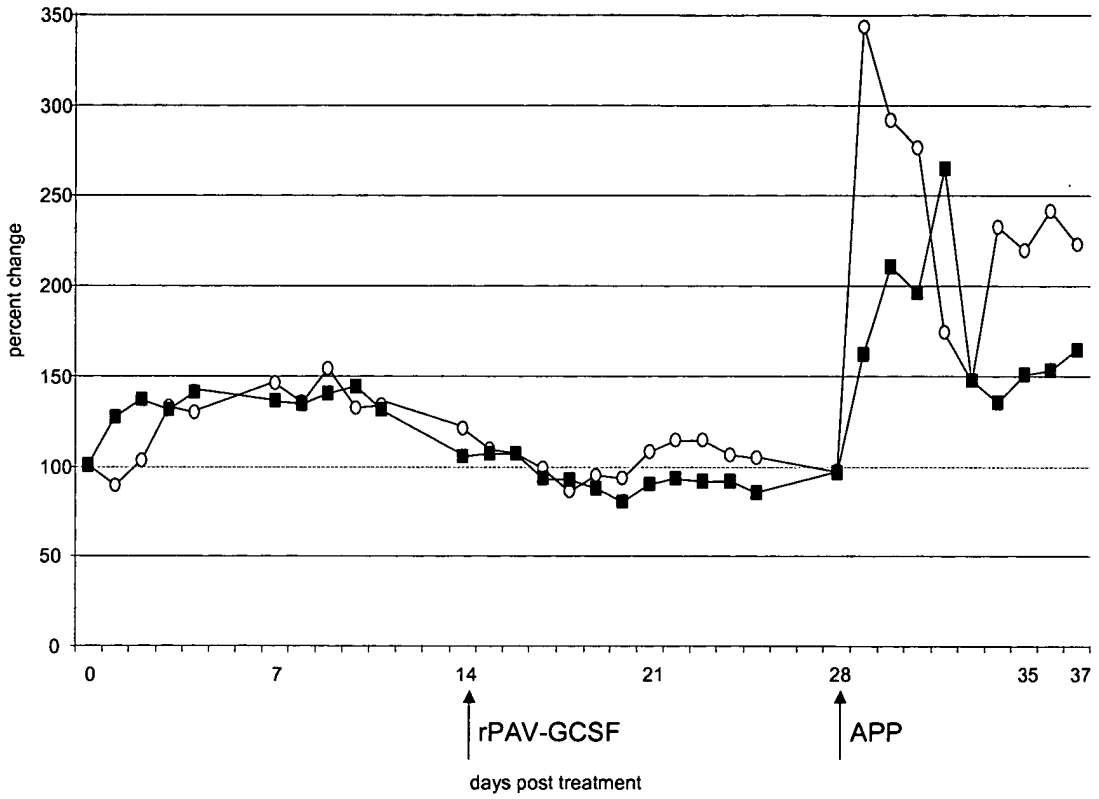


Figure 4: % Neutrophil change following a single treatment with rPAV-GCSF and challenge with APP.



Legend:
Filled squares, rPAdV-GCSF treated
Empty circles, untreated